ELSEVIER

Drug and Alcohol Dependence 65 (2002) 221–224

DRUG and ALCOHOL DEPENDENCE

www.elsevier.com/locate/drugalcdep

Association of a CB1 Cannabinoid Receptor Gene (*CNR1*) polymorphism with severe alcohol dependence

Lutz G. Schmidt [a,*], Jerzy Samochowiec [a,b,c], Ulrich Finckh [d], Ewa Fiszer-Piosik [b], Jan Horodnicki [b], B. Wendel [e], Hans Rommelspacher [c], Margret R. Hoehe [e]

[a] *Department of Psychiatry, University of Mainz, Untere Zahlbacher Straße 8, D-55131 Mainz, Germany*
[b] *Department of Psychiatry, Pomeranian Academy of Medicine, Broniewskiego 226, Pl-71-460 Szczecin, Poland*
[c] *Department of Clinical Neurobiology University Hospital Benjamin Franklin, Free University of Berlin, Ulmenallee 32, D-14050 Berlin Germany*
[d] *Department of Human Genetics, University Hospital Eppendorf, Butenfeld 42, D-22529 Hamburg, Germany*
[e] *Genome Research, Max–Delbrück-Center for Molecular Medicine, Robert Rössle-Strasse 10, D-13092 Berlin, Germany*

Received 13 June 2000; accepted 27 April 2001

Abstract

Due to the involvement of the endogenous cannabinoid system in brain reward mechanisms a silent polymorphism (1359G/A; Thr453Thr) in the single coding exon of the CB1 human cannabinoid receptor gene (*CNR1*) was analysed in 121 severely affected Caucasian alcoholics and 136 most likely non-alcoholic controls. The observed frequency of the A allele was 31.2% for controls and 42.1% for alcoholics with severe withdrawal syndromes ($P = 0.010$). Post-hoc exploration indicated that this allelic association resulted from an excess of the homozygous A/A genotype in patients with a history of alcohol delirium ($P = 0.031$, DF 2), suggesting an increased risk of delirium (OR = 2.45, 95% CI 1.14–5.25). This finding suggests that the homozygous genotype *CNR1* 1359A/A confers vulnerability to alcohol withdrawal delirium.

Keywords: CB1 Cannabinoid receptor gene (*CNR1*); Alcoholism; Alcohol withdrawal; Alcohol seizures; Genetics

1. Introduction

Alcohol dependence is a clinically and etiologically heterogeneous syndrome caused by a complex interaction of genetic and environmental factors (Merikangas, 1990). A high genetic load is expected in severely affected alcohol-dependent subjects (Lander and Schork, 1994) and as a consequence, alcoholics who display severe withdrawal symptoms are suitable for the genetic dissection of the complex genetic disposition to alcohol dependence (Noble et al., 2000).

Abnormalities in various neurotransmitter functions have been considered as the basis for withdrawal symptoms (Glue and Nutt, 1990), and genetic variants of the dopamine, serotonin, GABA, glutamate and opioid systems have been investigated in terms of how they might contribute to withdrawal vulnerability (Schmidt and Sander, 2000). The recently identified endogenous cannabinoid system (Devane et al., 1992)—characterized by the cloning (Matsuda et al., 1990) and mapping of the CB1 brain receptor gene (*CNR1*) (Hoehe et al., 1991)—was also shown to be part of the mesocorticolimbic reward pathway (Tanda et al., 2000). It has been demonstrated that the neurophysiological and pathological effects of chronic ethanol consumption might also be mediated through the endogenous cannabinoid system (Hungund and Basavarajappa, 2000a). Evidence for distinct differences in the binding characteristics of CB1 in the brain of mice selected for their differences in voluntary ethanol consumption (Hungund and Basavarajappa, 2000b) when combined with studies reporting genetic variants of *CNR1* associated with alcohol and drug addiction (Johnson et al., 1997),

* Corresponding author. Tel.: +49-6131-177335; fax: +49-6131-229974.
E-mail address: schmidt@psychiatrie.klinik.uni-mainz.de (L.G. Schmidt).

0376-8716/02/$ - see front matter
PII: S0376-8716(01)00164-8

(especially severe drug abuse; Comings et al., 1997), led us to the hypothesis that genetic variants of *CNR1* might have a significant effect on susceptibility to severe forms of alcohol dependence. In pilot studies (Hoehe et al., 1993; Gadzicki et al., 1999), we and others identified a silent transition polymorphism (1359G/A) at the third base of *CNR1* codon 453 (Thr) with population frequencies for the rarer allele (A) of 24–32% (Gadzicki et al., 1999; Hoehe et al., 2000). Therefore 1359G/A appeared to be suitable for case-control association studies. Because cannabis may act as a substitute in alcohol-withdrawal by counteracting symptoms such as tremor and nausea and because the use of both cannabis and alcohol may contribute to psychotic states (Childers and Breivogel, 1998), we restricted our analysis to subgroups of patients with a history of severe withdrawal symptoms (delirium or seizures.)

2. Methods

2.1. Subjects

The study protocol was approved by the Ethics Committee of the University Hospital Rudolf Virchow at the Free University of Berlin. Written informed consent was obtained from all participants. All alcohol-dependent patients and control subjects were unrelated individuals of German descent. The diagnostic assessment was performed without knowledge of the genotypic status.

2.2. Alcohol-dependent subjects

Our study included 121 Caucasian patients who fulfilled the criteria of the alcohol dependence syndrome according to the International Classification of Diseases, 10th rev., of the World Health Organization 1993. The mean ($\pm$S.D.) age of alcohol-dependent probands was 39.2 ($\pm$8.6) years. All patients were referred from the outpatient unit for addictive disorders at the Department of Psychiatry of the Free University of Berlin. Alcohol and substance abuse history was assessed by a structured interview that was based on the substance abuse section of the Composite International Diagnostic Interview (Robins et al., 1988). To isolate alcoholics with severe physiological withdrawal symptoms and a presumed substantial genetic load, we formed a subgroup of alcoholics on the basis of a history of alcohol-withdrawal seizures or delirium. We also formed two more homogenous subgroups—one with seizures but without delirium and one with delirium but without seizures (Schuckit et al., 1998).

2.3. Controls

A total of 136 Caucasian control subjects (with an average age of 42.2 ($\pm$9.2) years) were chosen from blood donors who had reported no history of addictive disorder or previous psychiatric treatment in a questionnaire.

2.4. DNA analyses

Genomic DNA was extracted from anticoagulated venous blood samples or lymphoblastoid cell lines using a salting out method (Miller et al., 1988). For an allele-specific polymerase chain reaction (PCR) to identify the *CNR1* 1359G/A-alleles (numbered according to Gerard et al., 1991), we used a pair of flanking primers (F1 and THAC-6R) together with two allele-specific internal primers. The allele-specific primers A3 and C2 detected the A-allele on the forward strand and the allele G on the complementary strand, respectively. The allele-specific primers T1 and G4 detected allele A on the complementary strand and the G-allele on the forward strand, respectively. Thus, one reaction tube contained the primers F1, THAC-6R, A3, and C2, and a second reaction tube contained primers F1, THAC-6R, T1, and G4 with the following sequences. F1: 5′-ACACATTGATGAAACCTACCTGAT-3′; THAC-6R: 5′-GGCGGCGGCAAATTCTTTTCCTGTGCTGCCAGGGAG-3′; A3: 5′-AGTGAGAGTTGCATCAAGAGCACA-3′; C2: 5′-GACTTGGCAATCTTGACC-3′; T1: 5′-GACTTGGCAATCTTGACT-3′; G4: 5′-AGTGAGAGTTGCATCAAGAGCAGC-3′. PCR reaction contained 10 ng of genomic DNA, 10 pmol of/for each primer, 50 mM KCl, 10 mM Tris–HCl (pH 8.3), 1.5 mM $MgCl_2$, 200 μM of each dNTP and 5 U *Taq* polymerase in a total volume of 21 μl. PCR was carried out in a Perkin Elmer 9600 thermocycler using the following PCR thermoprofile: 95 °C, 4 min; 12 × [94 °C, 50 s; 56 °C, 20 s; 72 °C, 30 s]; 23 × [94 °C, 50 s; 64 °C, 15 s; 72 °C, 30 s]; 72 °C, 10 min. Patterns of flanking PCR fragments (527 bp) and allele-specific bands (417/110 bp) were assessed by 2% agarose gel electrophoresis and ethidium bromide stain. Genotyping was carried out without knowledge of the sample status.

2.5. Statistical analyses

Genotype and allele frequencies, odds ratios (OR), and χ^2-tests were calculated using the SPSS computer program, release 8.0 (1998). Statistical analyses of deviations from Hardy–Weinberg equilibrium (HWE) were achieved with χ^2-tests. A Type I error rate of 5% was chosen for the analyses. A correction for multiple testing was not performed.

Table 1
Genotype counts and frequencies (%) in subgroups of severely affected alcoholics and controls

Subjects	Genotypes				χ^2-test[a]	P-value
	G/G	G/A	A/A	Total		
Controls	66 (0.49)	55 (0.40)	15 (0.11)	136		
Patients with severe alcohol withdrawal symptoms (i.e. seizures and/or delirium)	42 (0.35)	56 (0.46)	23 (0.19)	121	6.172	0.046
Patients with alcohol withdrawal seizures	26 (0.37)	36 (0.51)	9 (0.12)	71	2.715	0.257
Patients with alcohol delirium	25 (0.34)	31 (0.42)	17 (0.24)	73	6.935	0.031

[a] χ^2-test; df 2.

3. Results

The genotype counts for patients with severe withdrawal syndromes and controls are shown in Table 1. None of the genotype counts for the patients or controls deviated significantly from those expected according to the Hardy–Weinberg equilibrium. The allelic frequency of *CNR1* 1359A was 42.1% (102/242) in severely affected alcoholics in contrast to 31.3% (85/272) in 272 chromosomes of the controls ($\chi^2 = 6.572$, $P = 0.010$, DF = 1). Post-hoc exploration indicated that this allelic association resulted from a significant excess of the A/A genotype in patients with a history of alcohol delirium ($P = 0.031$, DF 2) and suggests an increased risk of delirium (OR = 2.45, 95% CI 1.14–5.25) in association with A/A homozygosity.

4. Discussion

The aim of the study was to investigate a phenotype–genotype relationship in subgroups of alcoholics with potential high genetic load attributable to a single gene effect. Due to the involvement of the endogenous cannabinoid system in reward mechanisms, and the role of cannabis and alcohol in inducing psychotic states, the genotype of the CB1 cannabinergic brain receptor polymorphism *CNR1* 1359G/A was assessed in alcoholics with severe forms of withdrawal. The result of our study suggests that the common intragenic *CNR1* polymorphism 1359G/A does confer vulnerability to alcohol-withdrawal delirium to some extent. Although the 1359G/A exchange is a silent mutation without an obvious effect on CB1 function, it has been investigated in association with various neuropsychiatric disorders; e.g. no association was found in patients with Gilles de la Tourette syndrome (Gadzicki et al., 1999).

There are several reasons why the present study is preliminary only. Firstly, it is unclear whether the observed association is due to alcohol dependence per se or to its withdrawal complication. Secondly, the case control design employed in this study may give rise to spurious associations if the described differences in allele frequency can be attributed to differences between the populations from which the clinical and the control samples were drawn. Further studies are therefore required to verify the tentative allelic and genotypic associations and a family-based study design would also be useful.

Acknowledgements

Jerzy Samochowiec gratefully acknowledges the receipt of a fellowship from the Alexander von Humboldt Stiftung. This work was supported by the Deutsche Forschungsgemeinschaft (He 916/7-2).

References

Childers, S.R., Breivogel, C.S., 1998. Cannabis and endogenous cannabinoid systems. Drug Alcohol Depend. 51, 173–187.

Comings, D.E., Muhleman, D., Gade, R., Johanson, P., Verde, R., Saucier, G., MacMurray, J., 1997. Cannabinoid receptor gene (CNR1): association with i.v. drug abuse. Mol. Psychiatry 2, 161–168.

Devane, W.A., Hanus, L., Breuer, A., 1992. Isolation and structure of a brain constituent that binds to the cannabinoid receptor. Science 258, 1946–1949.

Gadzicki, D., Müller-Vahl, K., Stuhrmann, M., 1999. A frequent polymorphism in the coding exon of the human cannabinoid receptor (CNR1) gene. Mol. Cell. Probes 13, 321–323.

Gerard, C.M., Mollereau, C., Vassart, G., Parmentier, M., 1991. Molecular cloning of a human cannabinoid receptor which is also expressed in testis. Biochem. J. 279, 129–134.

Glue, P., Nutt, D., 1990. Overexcitement and disinhibition. Dynamic neurotransmitter interaction in alcohol withdrawal. Br. J. Psychiatry 157, 491–499.

Hoehe, M.R., Caenazzo, L., Martinez, M.M., Hsieh, W.-T., Modi, W.S., Gershon, E.S., Bonner, T.I., 1991. Genetic and physical mapping of the human cannabinoid receptor gene to chromosome 6q14-q15. New Biol. 3 (9), 880–885.

Hoehe, M.R., Goldin, L.R., Martinez, M.M., Caenazzo, L., Hsieh, W.-T., Berrettini, W.H., Gershon, E.S., Bonner, T.I., Church, G.M., 1993. Molecular genetic studies of the human cannabinoid receptor gene. World Congress on Psychiatric Genetics, New Orleans. Psychiatr. Genet. 3, 132.

Hoehe, M.R., Rinn, T., Flachmeier, C., Heere, P., Kunert, H.J., Timmermann, B., Köpcke, K., Ehrenreich, H., 2000. Comparative sequencing of the human CB1 cannabinoid receptor gene (CNR1)

coding exon; no structural mutations in individuals exhibiting extreme responses to cannabis. Psychiatr. Genet. 10, 173–177.

Hungund, B.L., Basavarajappa, B.S., 2000a. Are anandamide and cannabinoid receptors involved in ethanol tolerance? A review of the evidence. Alcohol Alcohol. 35 (2), 126–133.

Hungund, B.L., Basavarajappa, B.S., 2000b. Distinct differences in the cannabinoid receptor binding in the brain of C57BL/6 and DBA/2 mice, selected for their differences in voluntary ethanol consumption. J. Neurosci. Res. 60 (1), 122–128.

Johnson, J.P., Muhleman, D., MacMurray, J., Gade, R., Verde, R., Ask, M., Kelley, J., Comings, D.E., 1997. Association between the cannabinoid receptor gene (CNR1) and the P300 event-related potential. Mol. Psychiatry 2, 169–171.

Lander, E.S., Schork, N.J., 1994. Genetic dissection of complex traits. Science 265, 2037–2048.

Matsuda, L.A., Lolait, S.J., Brownstein, M.J., Young, A.C., Bonner, T.J., 1990. Structure of a cannabinoid receptor and functional expression of the cloned cDNA. Nature 346 (6284), 561–564.

Merikangas, K.R., 1990. The genetic epidemiology of alcoholism. Psychol. Med. 20, 11–22.

Miller, S.A., Dykes, D., Polesky, H.F., 1988. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res. 16, 1215.

Noble, E.P., Zhang, X., Ritchie, T.L., Sparkes, R.S., 2000. Haplotypes at the DRD2 locus and severe alcoholism. Am. J. Med. Genet. 96 (5), 622–631.

Robins, L.E., Wing, J., Wittchen, H.U., Helzer, J.E., Babor, T.F., Burke, J., Farmer, A., Jablenski, A., Pickens, R., Regier, D.A., Sartorius, N., Towle, L.H., 1988. The Composite International Diagnostic Interview: an epidemiological instrument suitable for use in conjunction with different diagnostic systems and in different cultures. Arch. Gen. Psychiatry 45, 1069–1077.

Schmidt, L.G., Sander, T., 2000. Genetics of alcohol withdrawal. Eur. Psychiatry 15, 135–139.

Schuckit, M.A., Smith, T.L., Daeppen, J.B., Eng, M., Li, T.K., Hesselbrock, V.M., et al., 1998. Clinical relevance of the distinction between alcohol dependence with and without a physiological component. Am. J. Psychiatry 155, 733–740.

Tanda, G., Munzar, P., Goldberg, S.R., 2000. Self-administration behavior is maintained by the psychoactive ingredient of marijuana in squirrel monkeys. Nat. Neurosci. 3 (11), 1073–1074.